Racial differences in calcium retention in response to dietary salt

Dear Sir:

In a recent issue of the Journal, Wigertz et al (1) studied body calcium retention in black and white girls in response to changes in salt consumption. The authors used a randomized crossover design and tested a constant calcium intake of 815 mg/d (20 mmol/d) and 2 different intakes of dietary sodium: 1.30 g/d (57 mmol/d; low-sodium diet) and 3.86 g/d (168 mmol/d; high-sodium diet). They found that body calcium retention was significantly greater in black than in white girls and that body calcium retention was lower with the high-sodium diet than with the low-sodium diet. They suggested that a decrease in net intestinal calcium absorption was the main mechanism involved in the negative effect of the high salt load.

There is general agreement that high-sodium diets are associated with increased urinary calcium excretion, relative to low salt consumption (2). The white girls had the expected increase in urinary calcium excretion in response to a high-sodium diet, in line with previous reports. However, surprisingly, the black girls showed no such increase. The effect of changes in salt intake on intestinal calcium absorption and skeletal calcium uptake and release remains a controversial issue. In the present study, net intestinal calcium absorption decreased by 11% in response to a high-sodium diet in the black girls and by 3% in the white girls when measured with the fecal sampling method. In contrast, Breslau et al (3) found a 26% increase in fractional calcium absorption with the use of the isotope method.

Because of these discrepant findings, we examined carefully the calcium balance data for the young females studied by Wigertz et al (1: see Table 2). Calcium retention has been calculated as the difference between net calcium absorption (difference between calcium ingestion and fecal output) and urinary calcium excretion. As expected, the high-sodium diet induced an increase in urinary calcium excretion in the white girls but not in the black girls. However, sodium excretion was significantly higher in the white girls than in the black girls. In contrast, net intestinal calcium absorption decreased in the black girls but remained relatively constant in the white girls. When we recalculated calcium retention, based on the mean values indicated in Table 2 and a fixed calcium intake of 815 mg/d, we were surprised to end up with substantially different values for 3 of the 4 values in the bottom row of this table. Thus, we calculated a mean calcium retention of 431 mg/24 h (instead of 453 mg/24 h, as shown in Table 2) and of 250 mg/24 h (instead of 235 mg/24 h, as shown in Table 2), respectively, in the black and white girls who consumed the low-sodium diet and of 380 mg/24 h (instead of 359 mg/24 h, as shown in Table 2) and of 190 mg/24 h (instead of 189 mg/24 h, as shown in Table 2), respectively, in the black and white girls who consumed the high-sodium diet. Thus, the recalculation indicated a considerably lower than claimed difference in calcium retention in response to changes in dietary salt intake in the black girls (51 mg/24 h instead of the indicated 94 mg/24 h) and a slightly higher than claimed difference in the white girls (60 mg/24 h instead of the indicated 46 mg/24 h).

Finally, the finding by Wigertz et al (1) of a baseline sweat calcium excretion of 54 and 51 mg/24 h in the black and white girls, respectively, which is roughly the same amount as that excreted in urine under low-sodium conditions (50 and 53 mg/24 h, respectively), was unexpected as well. Although even higher calcium losses in sweat, namely 103 mg/d, were previously reported by this group of authors in healthy adult women (4), such high losses—to the best of our knowledge—have not been found by others. If correct, we wonder why the authors did not take into account sweat calcium excretion when they estimated body calcium retention and possible changes in response to changes in dietary salt intake in the black and white girls in their study (1), especially because changes in sodium load may lead to changes in sweat calcium loss.

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Reply to TB Drüeke and B Lacour

Dear Sir:

We thank Drüeke and Lacour for the opportunity to detail our analysis and expand on our methods. For each girl in our study, a daily diet composite was analyzed for calcium for almost all of the 42 d of the balance study. The mean (±SD) daily calcium intake was 815 ± 98 mg for the 78 analyzed diet composites. However, this mean calcium intake was not used in any of our calculations. For each girl, daily calcium absorption and retention were calculated on the basis of the analyzed daily calcium intake and fecal and urinary calcium excretion. Any food and beverages that were not consumed were analyzed, and the calcium from these foods was subtracted from the intake for that day for that subject. Daily calcium balances were calculated for each girl for the 42 d of the balance study. The range of daily calcium intake averaged over 42 d for each girl was 729–862 mg/d. The same technique was used to calculate daily sodium excretion. This explains the discrepancies between Drüeke and Lacour’s estimates of calcium absorption and retention and sodium excretion based on the mean values for the group and not on the daily values for the individual, which are provided in Table 2 of our article.

Drüeke and Lacour question why the 24-h dermal losses of calcium were not subtracted from the net retention values. Sweat calcium losses were measured on only one of the last days of each balance period. Because there were no significant effects of dietary salt intake or race on sweat calcium, as previously reported from this same study (1), we decided not to subtract the calcium sweat losses from a single 24-h period.

Although Drüeke and Lacour consider the 24-h sweat calcium losses of 51 and 54 mg/d to be high, we know of no studies other than our own that have estimated whole-body daily sweat calcium in adolescents. Estimation of sweat calcium in adults has been reported to vary from 0 to 149 mg/d under minimal sweating conditions (2). Using what is considered to be a more accurate method—whole-body 47Ca retention minus urinary and fecal 47Ca losses—Charles et al (3) calculated an average difference (assumed to be sweat calcium) of 63 mg/d in adults. The use of this method resulted in dermal calcium losses that were comparable with those we reported for adolescents; thus, we do not believe that our values are high.

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The association between plasma homocysteine and holo-transcobalamin and the transcobalamin 776C→G polymorphism is influenced by folate in the absence of supplementation and fortified diet

Dear Sir:

von Castel-Dunwoody et al (1, 2) recently reported that vitamin B-12 modulates the blood concentration of total homocysteine (tHcy) in a group of 359 nonpregnant young women who were previously characterized for folate and genetic determinants of tHcy. In our opinion, these interesting data may be reevaluated considering the folic acid fortification of grain products that was initiated in the United States (3). The high serum concentration and high dietary intake of folate may explain, at least in part, the significant association between tHcy and vitamin B-12. Quinlivan et al (4) showed that the usual dependency of homocysteine on folate diminishes and the vitamin B-12 concentration becomes the main determinant of tHcy in subjects who receive supplements with increasing doses of folic acid. Similarly, von Castel-Dunwoody et al (2) found a weak association between tHcy and folate but a stronger association between tHcy and vitamin B-12.

We evaluated the influence of folate on the association between tHcy and vitamin B-12 in a group of 114 adults from Western Europe who neither received supplements nor ate folate-enriched food. These subjects were studied in 2 previously published reports (5, 6). Compared with the group of von Castel-Dunwoody et al (1), these subjects had a higher plasma tHcy concentration (10.9 ± 3.4 compared with 6.3 ± 1.3 μmol/L), a much lower folate concentration (12.9 ± 4.6 compared with 50.2 ± 21.1 nmol/L), and no association between tHcy and vitamin B-12 (r = −0.093, P = 0.3272), despite a significant association between tHcy and folate (r = −0.217, P = 0.0253). When we stratified the association between tHcy and vitamin B-12 by quartiles of folate, it became significant only for the upper quartile (folate >15.0 nmol/L; r = −0.395, P = 0.0339). Similarly, we observed an association between tHcy and holo-transcobalamin only when folate concentrations were in the upper quartile (r = −0.394, P = 0.0379). In addition, the holo-transcobalamin concentration was highest in this upper quartile of folate (P < 0.0176), whereas no significant interaction was reported between quartiles of folate and either vitamin B-12 (P = 0.4644) or apo-transcobalamin (P = 0.7760). Therefore, these data suggest that the influence of folate on the association between tHcy and vitamin B-12 can be observed not only in subjects who receive either a fortified diet or vitamin supplements but also in those who have a relatively high dietary intake of folate.

A second interesting finding of von Castel-Dunwoody et al (1) was that the transcobalamin 776C→G polymorphism (P259R) negatively affected serum holo-transcobalamin, as previously
shown (7). In their study, the 259RR variants had a holo-
transcobalamin concentration that was 1.18-fold lower than
those of the 259PP variant, with a significant mean difference of
13 pmol/L. (1). We reported a 1.16-fold lower holo-
transcobalamin concentration in the 259RR variants than in the
259PP variants, but this small difference was not statistically
significant ($P = 0.2260$), possibly as the result of the limited size
of our sample population. In contrast, there was a clear-cut dif-
ference in apo-transcobalamin concentration, with a 1.59-fold
lower concentration in the 259RR variants ($\bar{x} \pm SD$: 250 pmol/L; range: 219–308 pmol/L) than in the 259PP and 259PR variants ($\bar{x} \pm SD$: 387 pmol/L; range: 317–463 pmol/L; $P < 0.0001$), as previously
reported (5). The greater influence of this polymorphism on
apo-transcobalamin concentrations, as opposed to holo-
transcobalamin, suggests a difference between the variants in the
vitamin B-12 binding affinity or blood half-life. However, when
stratifying the analysis by quartiles of vitamin B-12 concentra-
tions, the holo-transcobalamin concentration between the differ-
et genotypes was not influenced by vitamin B-12. Our group
was the first to report that the P259R substitution previously
observed in Caco-2 cells and HT 29 cells was a polymorphism
and to show its association with transcobalamin concentration in
cells and in blood (5, 8). We studied the molecular mechanisms
underlying the difference in the expression level of the variants.
We found that the RR variant was associated with a lower level
of transcripts than were the PR and PP variants and that the
isolectric point of the native and recombinant RR variant shifted
under both non-denaturing and denaturing conditions, which
suggested conformational changes. However, in contrast with the
references cited (7, 9) in the article by von Castel-Dunwoody et al (1), there is, to our knowledge, no direct evidence to support
the hypothesis that the 2 transcobalamin variants have different
vitamin B-12 binding affinities and different abilities to deliver
vitamin B-12 to cells. In our population (5, 6), we found that tHcy
was significantly higher in the 259PR heterozygous carriers (me-
dian: 11.5 pmol/L; interquartile range: 9.6–14.8 pmol/L) than in the
259PP and 259PR carriers (medians: 9.8 and 9.9 pmol/L,
respectively; interquartile ranges: 7.3–11.5 and 8.4–11.7
pmol/L, respectively; $P = 0.0015$). When stratifying the univar-
ate analysis by quartiles of plasma B-12, we found no influence
of vitamin B-12 on this association.

Because we showed that folate concentrations may influence
the association between tHcy and holo-transcobalamin, we aim
ed to investigate its influence on the association between
tHcy and the transcobalamin 776C→G polymorphism. Surpris-
ingly, we found that the polymorphism remained a significant
determinant of tHcy only in the subjects who had a folate concen-
tration in the lowest quartile. In this subset of the population, tHcy was highest in heterozygous variants (tHcy in the 259PR
variants: median: 12.2 pmol/L; interquartile range: 11.8–15.8;
tHcy in the 259PP and 259RR variants: median: 10.5 pmol/L;
interquartile range: 6.5–12.4 pmol/L; $P = 0.0009$). The associa-
tion between the transcobalamin polymorphism and tHcy was not related to transcobalamin concentrations, because the lowest concentrations were recorded in 259RR carriers. We previously suggested that the transcobalamin heterozygous
genotype may exhibit a lower affinity to the receptor than do the homozygous genotypes, but this needs to be confirmed by
direct binding experiments, because the functional properties
of the transcobalamin receptor are still a matter of debate (10).

The high folate concentration reported for the population stud-
ied by von Castel-Dunwoody et al (1) may therefore explain, at
least in part, why tHcy was not influenced by the transcobalamin
polymorphism, despite its association with holo-transcobalamin.
Indeed, the association between the transcobalamin 776C→G
polymorphism and tHcy that was reported in our subjects seemed
not to depend on the concentration of holo-transcobalamin.
Rather, it was influenced by low serum folate concentrations, at
least in a population that was exempted from a folate-fortified
diet or folate supplementation.

The authors had no conflicts of interest.

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In a recent issue of the Journal, Wannamethee et al (1) described the results of an interesting cross-sectional study on the association between anthropometric measures and metabolic abnormalities in 2924 men aged 60–79 y. They showed that body mass index and waist circumference had similar associations with cardiovascular disease risk factors, whereas the waist-to-hip ratio showed weaker correlations. We think that this conclusion could be misleading for several reasons. First, the authors’ definition of the metabolic syndrome is flawed; in their study, waist circumference was not included, and hypertension [blood pressure ≥140 (systolic)/90 (diastolic) mm Hg] was considered as one of the components of the metabolic syndrome phenotype, whereas the Adult Treatment Panel III definition of the metabolic syndrome includes elevated blood pressure [≥130 (systolic)/85 (diastolic) mm Hg], not hypertension (2). Therefore, the prevalence rate is not the true prevalence of the metabolic syndrome in this age category. This is why the prevalence of the metabolic syndrome was 14% by their definition, which is lower than that shown in elderly groups (3, 4). Second, the authors did not state that they had excluded subjects who were taking antihypertensive or lipid-lowering drugs. This could confound the findings. Third, the authors did not control for the effect of hip circumference in their analyses, but studies have shown that larger hip circumferences independently contribute to a reduced risk of metabolic abnormalities in adult and elderly men (5). Fourth, the odds ratio estimated from logistic regression models is a valid estimator of the rate ratio only when the outcome variable has a low prevalence in the sample (generally defined as ~10% or less); this is not the case for the metabolic abnormalities in this study, and, as the outcome condition becomes common, the odds ratio highly overestimates the rate ratio (6, 7). Fifth, a comparison of odds ratios is not a suitable method for judgments about the predictive ability of screening tools; a comparison of sensitivity, specificity, and accuracy between screening tools is recommended to reach this objective (8). Sixth, it is possible that the relation between each anthropometric measure and cardiovascular disease risk factors is mediated by another measure. Because the investigators did not control for the simultaneous effects of anthropometric measures, it is not clear which anthropometric measure has a higher correlation coefficient with metabolic risks. Seventh, the use of cutoffs for defining metabolic disorders implies a loss of information; therefore, the authors should have also assessed the relations between anthropometric variables and continuous metabolic variables by using a multiple linear regression.

The recognition of adiposity measures associated with metabolic abnormalities is extremely important, because the prevention of these risk factors is of public health importance for the prevention of non-communicable diseases. However, careful epidemiologic and statistical methods should be adopted to avoid any incorrect conclusions.

None of the authors had any conflicts of interest.

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Reply to Esmaillzadeh et al

Dear Sir:

Esmaillzadeh et al (1) recently published a study in Iranian men, which were much younger than those in our study, that claims that the waist-to-hip ratio (WHR) is a better marker of metabolic abnormalities than are body mass index (BMI) or waist circumference (WC). In their letter, they make a number of criticisms on the methods used in our article. Here, we respond to their main concerns.

Prevalence of metabolic syndrome

If our aim had been to estimate the prevalence of the metabolic syndrome in the population, then our definition of WC would not yield the correct prevalence. However, this article was not about prevalence per se. A major purpose of our article was to investigate the association between the different adiposity measures and metabolic abnormalities and to compare the predictive abilities of the different adiposity measures in the detection of metabolic abnormalities. Therefore, it would have been inappropriate to include WC as part of the metabolic syndrome definition. This would inevitably have led to a correlation between metabolic abnormalities and WC that would have been greater than those between metabolic abnormalities and the other indexes that we investigated. We stated this clearly in the Methods section but retained the term “metabolic syndrome,” which is comparable with the definition used in a recent study that compared the association between WC and BMI and the metabolic syndrome (2).

Hypertension and lipid-lowering drugs

The results were not dependent on the definition of hypertension used; repeating the analyses with different definitions of hypertension did not affect the results. In our article, we stated...
that the definition of hypertension included men taking antihypertensive drugs. Although we did not specifically exclude men taking lipid-lowering drugs, men with a history of ischemic heart disease and diabetes (which accounted for most of the study subjects who were taking lipid-lowering drugs) were excluded. Our findings are thus highly unlikely to be confounded by men who were taking antihypertensive or lipid-lowering drugs.

**Rate ratio**

The rate ratio would not be appropriate for this analysis because the data were cross-sectional. Whereas the odds ratio derived from a logistic regression would be an overestimate of the risk ratio, the ranking of risk ratios obtained for the various anthropometric indexes would not be affected, so that the BMI and WC would still emerge as the best predictors.

**Sensitivity and specificity**

Sensitivity and specificity are useful measures of quantifying the predictive ability of anthropometric measurements, which were calculated and displayed in Figures 1 and 2 of our article in the receiver operating characteristic curves, which display sensitivity and specificity for a range of cutoffs. These analyses clearly showed that BMI and WC were the best predictors. The odd ratios usefully summarize these differences in predictive ability.

**Cutoffs**

The metabolic syndrome is used as a dichotomous variable, and the components are based on defined cutoffs. For clinical purposes, this is a more useful indicator of dyslipidemia or hypertension. We therefore analyzed our data as such. We do not believe that treating the metabolic abnormalities score as a quantitative variable would change the rankings of strengths of relations for the various adiposity indexes. Indeed, this would prevent our calculation of sensitivity and specificity (see above).

**Waist-to-hip ratio**

The major purpose of the article was to assess which measure (or measures) of adiposity would be most useful to identify men with metabolic abnormalities in clinical practice. We compared the waist-to-hip ratio with BMI and WC, and our data strongly suggested that WHR was neither as sensitive nor as specific as WC or BMI in these elderly men.

The authors had no conflicts of interest.

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**Erratum**


In Figure 2, the units for intact parathyroid hormone should be pg/mL rather than pg/L.